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Pharmacological effects of ethanol extract of Egyptian *Artemisia herba-alba* in rats and mice

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ABSTRACT

Objective: To investigate some pharmacological effects including gastroprotective, anti-inflammatory, analgesic, antipyretic and *in vitro* antioxidant effects of *Artemisia herba-alba* extract in different experimental models.

Methods: Inflammation was induced in rat paw by subcutaneous injection of 1% (v/v) carrageenan solution. Writhes was induced in mice by intraperitoneal injection of 0.6% (v/v) acetic acid solution. Pyrexia was induced using Brewer's yeast suspension. Gastric lesion was induced in rats by oral administration of 99% ethanol. The anti-inflammatory, analgesic, antipyretic and gastroprotective activities of *Artemisia herba-alba* extract were investigated respectively. *In vitro* antioxidant effect was investigated using DPPH free radical.

Results: The plant extract showed anti-inflammatory effect in carrageenan-induced paw edema in rats, analgesic effect against acetic acid-induced writhing, and antipyretic activity in Brewer's yeast model of pyrexia. Besides, it was shown to be a gastroprotective agent against ethanol-induced gastric ulcers. The plant also exhibited a free radical scavenging potential in an *in vitro* antioxidant study using DPPH.

Conclusions: The results validate the use of the investigated plant in traditional medicine for different ailments.

1. Introduction

Natural products have contributed greatly to the development of modern therapeutic drugs over the years. Plants represent various natural sources of useful compounds that might serve as lead for the development of novel drugs. Drugs of herbal origin are frequently considered to be less toxic and induce fewer side effects than synthetic ones [1]. Hence, pharmacological research on phytochemicals has become mandatory to establish the claimed medicinal properties of herbs [2]. *Artemisia herba-alba* (*A. herba-alba*) popularly known in Egypt as "Sheh", is a well-known medicinal plant that has been used in the Middle East traditional medicine for treating various diseases. It is used by local population of some countries as an anti-diabetic [3,4]. Herbal infusions from this species have been used as analgesic, antibacterial, and hemostatic agents [5,6]. It is used in Jordon in the form of a decoction against fever, menstrual and nervous problems [7]. The essential oil of this herb was found to be responsible for its therapeutic use as disinfectant, anthelmintic and antispasmodic [6].

Despite its wide traditional use, few systemic experimental studies were carried out to affirm traditional use of *A. herba-alba*. In this context, the present work was established to evaluate some pharmacological effects including gastroprotective, anti-inflammatory, analgesic, antipyretic and *in vitro* antioxidant effects of this herb in different animal models.

2. Materials and methods

2.1. Plant material

A. herba-alba belongs to family Asteraceae. The dried aerial parts of the plant were purchased from the Egyptian markets and were grinded by electric grinder.

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2.2. Preparation of plant extract

The plant powder was soaked in 70% ethyl alcohol for about 3 days, filtered using filter paper. The filtrate was concentrated under vacuum using the rotating evaporator (Rotavap), then percolated several times till exhaustion. The yielded ethanolic extract of *A. herba-alba* (55 g out of 200 g dried powder) was ready for both toxicological and pharmacological studies.

2.3. Animals

Healthy male Wister rats, weighing 130–160 g, and male Swiss albino mice, weighing 20–25 g, were obtained from the animal house of the National Research Centre. Before initiating the experiments, the rats or mice were allowed to acclimatize for few days under standard environmental conditions (12 h dark/12 h light cycle; temperature 20–22 °C; relative humidity 40%–60%). The study was conducted according to regulations of the ethics committee of the National Research Centre which gave its consent in accordance with the National Regulations on Animal Welfare and Institutional Animal Ethical Committee.

2.4. Drugs and chemicals

Indomethacin was obtained from Egyptian International Pharmaceutical Industries Company, and used at dose of 10 mg/kg as standard anti-inflammatory and analgesic drug. Paracetamol was purchased from Memphis Co., Egypt, and used at a dose of 150 mg/kg [8]. The drugs were given orally by gastric tube. Carrageenan and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Co., USA; whereas, ethyl alcohol was purchased from BDH-Chemical, England.

2.5. Experimental design

2.5.1. Determination of median lethal dose (LD_{50})

Five groups of six rats each received the plant extract in doses ranging from 1 to 4 g/kg body weight. The toxic symptoms, mortality rate, and post-mortem findings in each group were recorded 24 h after administration. The LD_{50} of the tested extract was calculated according to the following formula:

$$LD_{50} = \frac{Dm - \sum (z \times d)}{N}$$

where, Dm means the largest dose that kills all animals; z means the mean of dead animals between 2 successive groups; d means the constant factor between 2 successive doses; N means the number of animals in each group; $\sum$ means the sum of z × d.

One tenth, one twentieth and one fortieth of the maximum dose (4 g/kg body weight) of the plant extract that did not cause mortalities or toxic symptoms in rats were chosen to be used for the biological investigation throughout the study.

2.5.2. Acute anti-inflammatory test

The acute anti-inflammatory effect of the ethanolic extract was evaluated in the carrageenan-induced rat hind paw edema model [9].

A total of 30 adult male rats were divided into 5 groups of 6 animals each. The first group served as control and received normal saline. The second group was administered with indomethacin (10 mg/kg *p.o.*) as the standard anti-inflammatory drug. The third, fourth and fifth groups received the ethanolic extract of *A. herba-alba* at 100, 200 and 400 mg/kg *p.o.*, respectively. One hour after the oral administration of the extract, all the animals were injected with 0.1 mL of 1% (v/v) carrageenan solution in saline subcutaneously at the sub-planter area of the right hind paw. The paw volume of each rat was measured using planimeter before carrageenan injection and then followed by hourly measurement up to 4 h post carrageenan administration. The percent of volume of formed edema in each group were calculated as follows:

$$\text{Edema}(\%) = \frac{V_t - V_o}{V_o} \times 100$$

where, V_o is the paw volume before carrageenin injection (mL); V_t is the paw volume at t hour after carrageenin injection (mL).

Percent of inhibition of paw edema was also calculated as follows:

$$\%\ \text{Inhibition} = \frac{E_c - E_t}{E_c} \times 100$$

where, E_c is the edema of control group; E_t is the edema of *A. herba-alba* extract treated group.

2.5.3. Antinociceptive activity (writhing test)

A total of 30 adult male albino mice were divided into 5 groups of 6 animals each. The first group served as control and received normal saline. The second group was administered with indomethacin (10 mg/kg *p.o.*) as the standard analgesic drug. The third, fourth and fifth groups received oral administration of 100, 200 and 400 mg/kg of *A. herba-alba* ethanolic extract, respectively. Thirty minutes later, all groups were given 10 mL/kg intraperitoneal injection of 0.6% (v/v) acetic acid solution [10]. Five minutes after acetic acid injection, the number of writhes like abdominal muscle contraction, stretching of the hind limbs and trunk twisting were counted for 20 min. Percentage protection against writhes was taken as an index of analgesia and calculated as:

$$\frac{\text{No. of writhes in control group} - \text{No. of writhes in treated group}}{\text{No. of writhes in control group}} \times 100$$

2.5.4. Antipyretic activity (Brewer's yeast test)

In this experiment, the Brewer's yeast suspension was used to induce fever in all the rats [11]. Body temperature of each animal was measured from the rectum using digital thermometer and recorded before yeast injection. Each animal was then injected intramuscularly with pyrogenic dose of Brewer's yeast (1 mL/100 g body weight of 44% yeast suspension in saline). The rectal temperature measured 18 h following the yeast injection was considered as the basal line of elevated body temperature, based on which the antipyretic effect will be compared. Rats expressing >0.3 °C increase in rectal temperature were considered pyretic and selected to complete the experiment. Thirty male rats were randomly allocated into 5 groups, one group received saline as control; second group received paracetamol (standard antipyretic drug) at a dose of 150 mg/kg, and the remaining three groups received *A. herba-alba* extract at doses of 100, 200, and 400 mg/kg, respectively. A single oral administration of the tested extract, paracetamol [12]

or saline (control) was carried out and the rectal temperature was determined after 30, 60, and 120 min of intervention.

2.5.5. Gastroprotective activity against ethanol-induced ulcers

Rats were divided into five groups (6 rats per group). One group received saline as control; the second group received ranitidine (50 mg/kg) and the remaining groups received the ethanolic extract of *A. herba-alba* (100, 200 and 400 mg/kg). One hour later, gastric lesion was induced in rats by orally giving 1 mL ethanol (99%) in accordance to method described by Shabanah [13]. Rats were sacrificed one hour after ethanol administration by cervical dislocation after being lightly anaesthetized with ether. Then the stomach was excised, opened along the greater curvature, rinsed with saline, extended on a plastic board and examined for mucosal lesions. The number of lesions per rat were determined and noted as ulcer number. Gastric lesions were also scaled and scored according to their severities between 1 and 5 as follows: 1 for petechial lesions, 2 for lesions less than 1 mm, 3 for lesion between 1 and 2 mm, 4 for lesions between 2 and 4 mm, 5 for lesions more than 4 mm. Total ulcer score/rat was calculated by dividing the total ulcer score (given according to lesion severity) by the number of all lesions for each rat. Data are expressed as mean ulcer number or total ulcer score ± SEM for each group of animals [14].

2.5.6. In vitro antioxidant activity study using DPPH

The antioxidant activity of the investigated extract, based on the activity to scavenge the stable DPPH free radical, was determined by the method described by Braca *et al.* [15]. Plant extract (0.1 mL) in different concentrations (5–100 mg/mL) was added to 3 mL of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition was calculated by the formula: $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control (DPPH alone), and A_1 is the absorbance of the extract/standard (ascorbic acid). IC_{50} value was determined from the graph of percentage of inhibition plotted against the log concentration of the extract using GraphPad Prism Software version 6.0. IC_{50} is defined as the concentration of extract needed to inhibit 50% of DPPH radicals.

2.6. Statistical analysis

Statistical analysis for all tests (except gastric ulcer number and severity) was carried out using One-way ANOVA followed by Tukey *post hoc* test using SPSS software, version 14.0 (SPSS Inc., Chicago, Illinois, USA). For gastric ulcer number and severity tests, statistical significance was determined by Kruskal–Wallis non-parametric One-way ANOVA followed by Mann–Whitney multiple comparisons test. Data were represented as mean ± SEM. The P values less than 0.05 were considered to be significant.

3. Results

3.1. Evaluation of the acute anti-inflammatory effect

The edema model was established successfully in the hind paw of rats using 0.1 mL of 1% carrageenan. Figure 1 shows

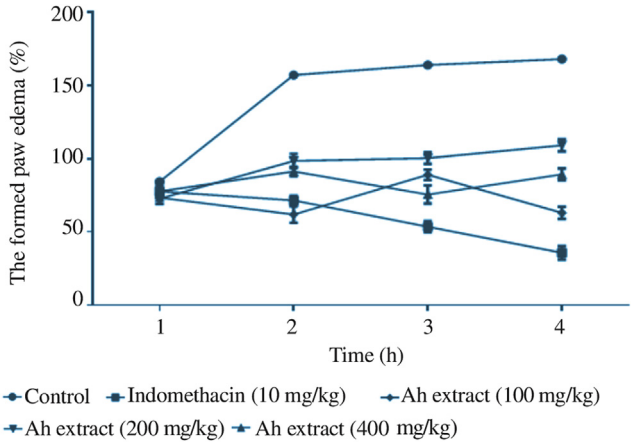

Figure 1. Effect of ethanolic extract of *A. herba-alba* on carrageenan-induced paw edema in rats.
Each value represents mean % volume of paw edema ± SEM (n = 6). Statistical analysis was carried out by One-way ANOVA followed by Tukey *post hoc* test. Ah: *A. herba-alba*.

that pretreatment with the *A. herba-alba* extract resulted in significant ($P < 0.05$) reduction in paw volume starting from the second hour after carrageenan injection as compared to the control group. *A. herba-alba* extract at doses of 400, 200 and 100 mg/kg exhibited % inhibition of paw edema by 46.8%, 35.0% and 62.5% at the end of the experiment (the forth hour) respectively. However % inhibition of paw volume was less than that of standard drug, indomethacin (78.8%).

3.2. Evaluation of the antinociceptive activity

Writhes was induced successfully in all mice by acetic acid injection represented by subsequent abdominal muscle contraction and stretching of hind limbs. As shown in Table 1, indomethacin (10 mg/kg) inhibited writhes by 91.9%. *A. herba-alba* extract significantly ($P < 0.05$) decreased the number of acetic acid-induced writhes. It showed writhes inhibition of 98.1%, 52.0% and 22.5% at doses of 100, 200 and 400 mg/kg.

Table 1
Effect of ethanolic extract of *A. herba-alba* on acetic acid-induced writhing in mice.

Treatment	No. of writhes (mean ± SEM)	% Inhibition
Control	54.2 ± 2.4	–
Indomethacin (10 mg/kg)	4.4 ± 0.5*	91.9
Ah extract (100 mg/kg)	1.0 ± 0.0*	98.1
Ah extract (200 mg/kg)	26.0 ± 0.7*	52.0
Ah extract (400 mg/kg)	42.0 ± 1.4*	22.5

*: Significantly different from control group at $P < 0.05$. Statistical analysis was carried out using One-way ANOVA test followed by Tukey *post hoc* test. Ah: *A. herba-alba*.

3.3. Evaluation of antipyretic activity

As shown in Figure 2, subcutaneous injection of Brewer's yeast-induced pyrexia in all rats 18 h after administration. Administration of the ethanolic extract of *A. herba-alba* at a dose of 100 mg/kg significantly ($P < 0.05$) decreased rectal temperature [(36.60 ± 0.27) °C] as compared to the yeast control group [(38.00 ± 0.16) °C] after 120 min of induced

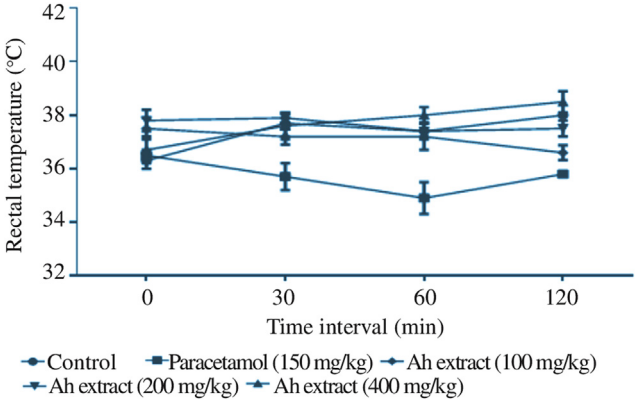

Figure 2. Effect of ethanolic extract of *A. herba-alba* on Brewer's yeast-induced pyrexia in rats.
Each value represents mean rectal temperature ± SEM (n = 6) compared to control group. Statistical analysis was carried out by One-way ANOVA followed by Tukey *post hoc* test. Ah: *A. herba-alba*.

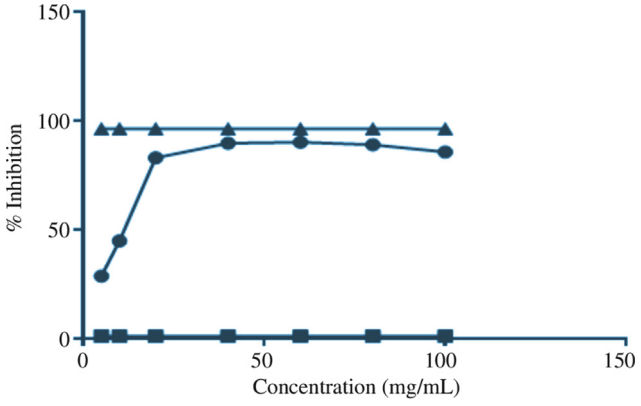

Figure 3. Scavenging activity of DPPH free radical by different concentrations of ethanolic extract of *A. herba-alba* or ascorbic acid.
Ah: *A. herba-alba*.

pyrexia. The antipyretic effect exhibited by the extract was comparable to that of the standard drug, paracetamol [(35.80 ± 0.12) °C].

3.4. Evaluation of gastroprotective effect

Pretreatment of the *A. herba-alba* extract suppressed the ethanol-induced gastric lesions. Administration of the extract at the doses of 100, 200 and 400 mg/kg decreased number of ulcers (6.6 ± 0.5, 7.4 ± 0.5 and 6.6 ± 0.5, respectively) compared to control group (24.6 ± 1.2). Administration of the same doses of the extract reduced ulcer severity by 82%, 76% and 79%, respectively compared to group pretreated with standard ranitidine (95%) (Table 2).

Table 2
Effect of ethanolic extract of *A. herba-alba* on gastric ulcers induced by 99% ethanol in rats.

Treatment	Ulcer number	Total ulcer score
Control	24.6 ± 1.2	81.8 ± 2.0
Ranitidine (50 mg/kg)	2.4 ± 0.2*	3.8 ± 0.4*
Ah extract (100 mg/kg)	6.6 ± 0.5*	14.4 ± 0.9*
Ah extract (200 mg/kg)	7.4 ± 0.5*	19.2 ± 0.6*
Ah extract (400 mg/kg)	6.6 ± 0.5*	17.2 ± 0.6*

All groups received *p.o.* administration of 99% ethanol (1 mL/rat) 1 h after drug administration. Each value represents the mean gastric ulcer number or total ulcer score ± SEM (n = 6). *: Significantly different from control group at $P < 0.05$. Statistical analysis was carried out by Kruskal–Wallis non-parametric One-way ANOVA followed by Mann–Whitney multiple comparisons test. Ah: *A. herba-alba.*

3.5. Assessment of in vitro antioxidant activity using DPPH

A. herba-alba extract and standard (ascorbic acid) showed a significant increase in the inhibition of DPPH radicals (Figure 3). Free radical scavenging activity also increased with increasing concentrations of the extract in the range of 5–100 mg/mL with IC_{50} value of (14.91 ± 0.16) mg/mL.

4. Discussion

The present study clearly demonstrates the anti-inflammatory effect of *A. herba-alba* indicated by inhibition of paw edema formation in rats. Edema due to carrageenan injection is formed *via* enhancement of inflammatory mediators that increase vascular permeability and/or increase blood flow [16], and includes two phases. The early phase is related to the production of histamine, serotonin, cyclooxygenase products and kinin-like substances; whereas, the second phase is mainly due to the release of prostaglandins, free radicals, proteases, and lysosomes [17]. *A. herba-alba* extract exhibited its anti-inflammatory effect *via* suppression of rat hind paw edema in the later phase after two hours of injection of the phlogistic agent. Thus, the effect may be presumed to be due to the influence of the extract on the inflammatory mediators and also on the pathway of prostaglandins synthesis. *A. herba-alba* is a rich source of flavonoids such as hispidulin and cirsilineol. Flavonoids isolated from some medicinal plants have been proven to possess anti-inflammatory effect [18]. It is therefore possible that the anti-inflammatory effect observed within this extract may be attributable to its flavonoid component.

The inflammatory response has been associated with various manifestations such as pain and elevated body temperature. The ethanolic extract of *A. herba-alba* also showed analgesic activity in writhing model of peripheral algesia in mice. Abdominal constriction response induced by acetic acid is a well-known sensitive procedure to evaluate peripherally acting analgesics [19]. Injection of acetic acid induces indirect release of prostaglandins and lipooxygenase products which stimulate the nociceptive neurons sensitive to the non-steroidal anti-inflammatory drugs [20]. The current results suggest that the extract may act by inhibition of lipooxygenase and/or cyclooxygenase in the peripheral tissues, hence, interfering with the synthesis or action of prostaglandins. The antipyretic activity was tested using Brewer's yeast-induced hyperthermia in rats. Yeast induces a kind of pathogenic fever which involves production of prostaglandins. Pro-inflammatory mediators such as cytokines and tumor necrosis factor are consequently released and increase the synthesis of prostaglandin E2 near preoptic hypothalamus area, thereby triggering the hypothalamus to elevate the body temperature [21]. Flavonoids have been documented to exert antipyretic effect *via* inhibition of

prostaglandin synthase [22]. *A. herba-alba* extract at a dose 100 mg/kg decreased rectal temperature 2 h after treatment in a similar manner to the standard drug paracetamol. The antipyretic effect of *A. herba-alba* was not reported previously and the mechanism of action is unknown. However, it seems that the antipyretic potential may be ascribed to the flavonoid constituent of the herb.

Gastric ulcer induced by ethanol is a widely used experimental model for evaluation of gastroprotective activity. Ethanol is known as a damaging agent to the stomach that acts by a direct necrotizing action, which in turn reduces bicarbonate secretion and mucus production. Gastric damage caused by ethanol may be due to the generation of reactive species, decreased cell proliferation, and an exacerbated inflammatory response [23]. The present results show that the ethanolic extract of *A. herba-alba* could protect against gastric lesions and ulcers induced by ethanol administration. Flavonoids and phenolic compounds are well known for the antiulcer activity, and were found to be active in this experimental model producing anti-ulcerogenic effect [24]. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion [25]. In addition, the polyphenol-rich compounds could protect erythrocytes from oxidative damage [26]. Polyphenols from strawberry extracts showed an important gastroprotective effect against ethanol-induced gastric damage due to their ability to maintain the cell membrane integrity, reduce the free radical-dependent lipid peroxidation and preserve and/or activate endogenous antioxidant enzymes. All these features help to protect gastric mucosa from oxidative damage and to strengthen the mucosa barrier, the first line of defense against exogenous damaging agent [27]. Therefore, the observed ulcer curative activity of *A. herba-alba* extract may be partially due to the relative antioxidant activity of its phytochemicals.

DPPH is stable nitrogen centered free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. It is a precise and reliable model for evaluation of free radical scavenging activity and detecting the antioxidant potential of several compounds [28]. The results of the present study reveal that the ethanolic extract of *A. herba-alba* exhibits a powerful free radical scavenging property in the DPPH model in a concentration dependent manner. The presence of phenolic compounds such as flavonoids, polyphenols and terpenes in *A. herba-alba* may contribute to its antioxidant effect. Studies have shown that these phytochemicals protect against glutathione depletion and increase the capacity of antioxidant enzymes. Phenolic compounds are understood to induce the cellular antioxidant system and increase approximately 50% cellular glutathione concentrations [29]. Oxidative stress occurs when free radical formation exceeds the body's ability to protect or scavenge them and forms the pathological basis of several chronic disease conditions. Hence, therapy using free-radical scavenging antioxidants has potential to prevent or ameliorate many of these disorders [30].

The present results extend the potential traditional use of *A. herba-alba* in folk medicine. Our data support the reasons for therapeutic use of this plant for treatment of inflammation, and the associated disorders like pain and pyrexia, and its use as gastroprotective agent. It can be concluded that *A. herba-alba* act, in part, through its antioxidant effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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